Distribution of phosphorus in roots and nodules of leguminous plant by X-ray fluorescence

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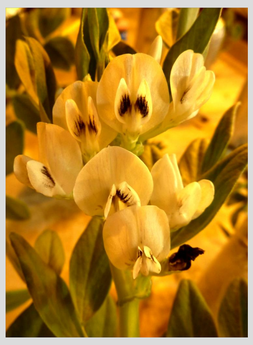


Vicia faba inflorescence

Symbiotic nitrogen fixation is a highly energy consuming process which occurs in **legume nodules**, organs that develop on the roots of legumes, and **host rhizobia, the bacteria capable of atmospheric nitrogen (N_2) fixation**. The objective of this study is to better understand **phosphorus (P) utilization** for N_2 fixation by nodules, by comparing elemental P distribution in roots and nodules of legumes grown under sufficient (**Psuf**) or deficient (**Pdef**) **P supplies**. **Micro-X-ray fluorescence (*μ*-XRF)** technique is used for P mapping, together with other elements such as Cl, S, Mg, Na, on cryo thin sections.

1. Plant growth conditions

The study was set up with ***Vicia faba*** (CV Diva), cultivated in a glasshouse under controlled conditions:

- 30/20°C day/night temperature
- 16 h photoperiod
- 70 % relative humidity during the day.

Psuf = 125 µmol of KH_2PO_4

Pdef = 25 µmol of KH_2PO_4

per plant per week

One week after sowing, the plantlets were individually transferred into 1 L serum bottle containing nutritive solution [1], and **inoculated with a rhizobia (Fb2).**

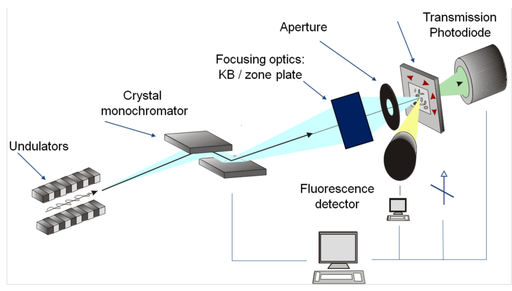
Plants grown in hydro-aeropony

2. Samples preparation and XRF maps collection on ID21 beamline (ESRF)

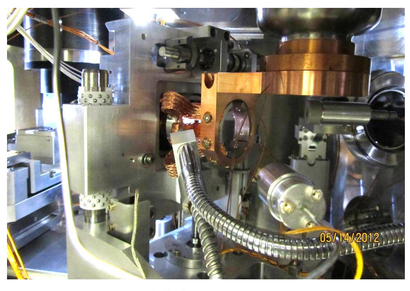
Before harvesting the plants at flowering stage (8 weeks after transfer), nodules and roots segments (1 cm above the apex), were picked-up, immediately immersed in cryo embedding resin-compound and flash frozen using methyl butane cooled by liquid N_2.

Thin sections 20 µm (roots) or 30 µm thick (across infected zone for nodules) were prepared with a **cryomicrotome** at the ID21 beamline [2], and transferred into the X-ray microscope, equipped with a **cryostat**.

X-ray fluorescence maps were collected with a focused beam of 0.5 (v.) × 0.8 (h.) µm^2 on the cryo thin sections, using an excitation energy of **3 keV** to collect simultaneously **Cl, S, P,** Si, Al, Mg and Na elemental maps



ID21 microspectrocopy beamline

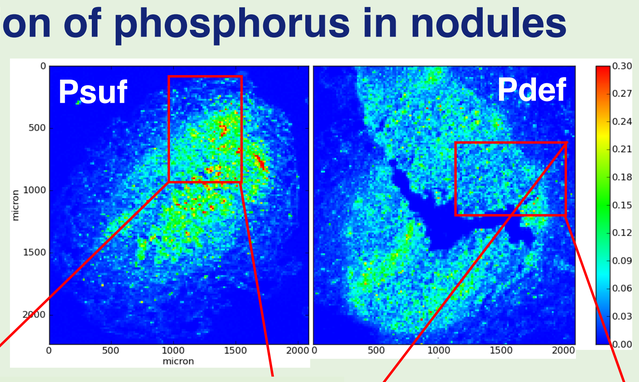


ID21 cryostage

3. Distribution of phosphorus in nodules

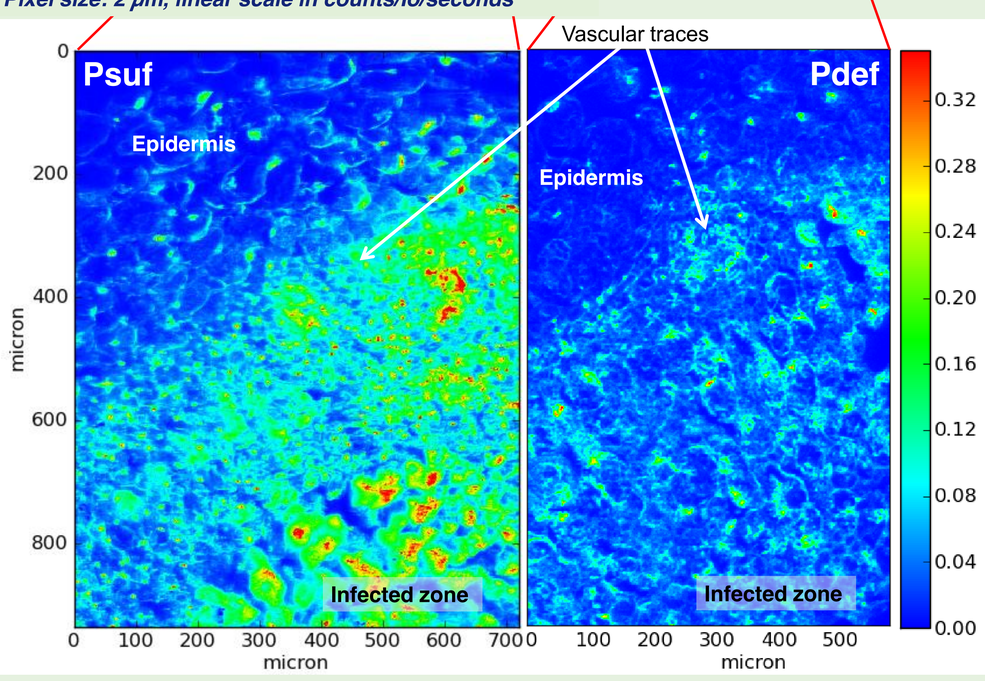
Entire nodules

Pixel size = 15 µm, the linear scale is in counts/Io/seconds, Io being the value of the incident flux



Enlargement of the red rectangles

Pixel size: 2 µm, linear scale in counts/Io/seconds

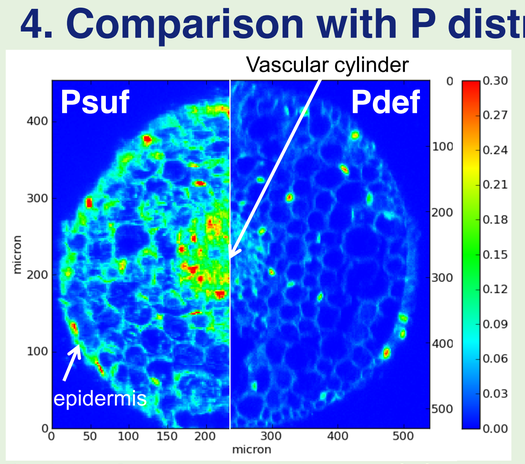


Lower amount of P in the epidermis than in the central part of the nodules.

Comparison between Psuf and Pdef nodules:

- Global decrease of P amount in Pdef nodule compared to Psuf and more pronounced in the infected zone.
- Vascular traces better underlined by P–rich pixels in Pdef than in Psuf.

4. Comparison with P distribution in roots



Pixel size: 2 µm, linear scale in counts/Io/seconds

Higher amounts of P in cells walls, cells cores and vascular cylinder than in the other constituents of the root.

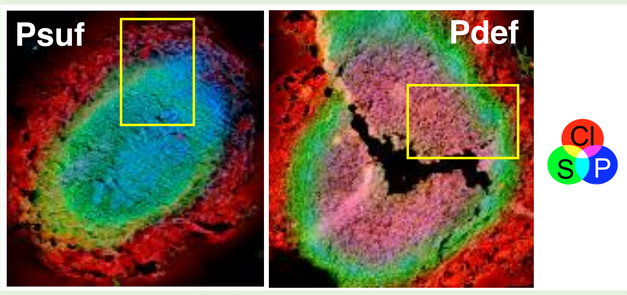
Comparison between Psuf and Pdef:

- Decrease of P amount in Pdef roots compared to Psuf one, more pronounced in the epidermal cells.

Comparison between roots and nodules:

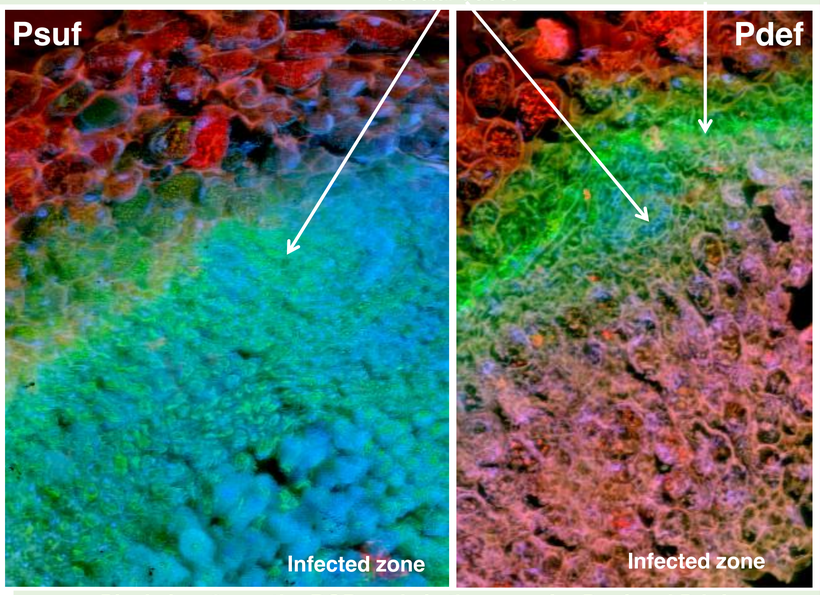
- No particular P-impoverishment of the epidermis for the nodules, in contrast to root.
- Vessels underlined by P–rich pixels in Psuf root whereas better underlined by P–rich pixels in Pdef nodules.

5. Distribution of the other elements

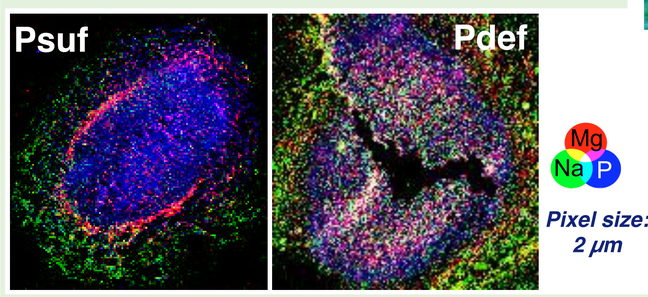


Pixel size: 15 µm
The RGB scale is the same for Psuf and Pdef

- **S** mainly localized in and around the nodules endodermis, and more marked in Pdef than in Psuf.
- **P** decrease in the infected zone of Pdef correlated with a **Cl** increase.



Pixel size: 2 µm, the RGB scale is the same for Psuf and Pdef



Pixel size: 2 µm

The RGB scale is the same for Psuf and Pdef

- **Mg** preferentially located in the nodule endodermis for Psuf whereas Mg more homogeneously distributed in the whole tissues of Pdef nodule.
- **P decrease** in the infected zone of Pdef correlated with **Na and Cl increase**.

5. Conclusions and perspectives

P µ-XRF maps → The P amount in the nodules was affected by the amounts of P supplied to the plant. The P distribution differed from the one observed in the roots.

other µ-XRF maps → The distribution of S, Cl, Mg and Na was also affected by the amounts of P supplied to the plant.

- Explain the effect of P supplies on Cl and Na increase under Pdef.
- Check for the reproducibility of the distribution by collecting additional XRF maps on other Psuf and Pdef nodules.

References

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2. M. Salomé, M. Cotte, R. Baker, R. Barrett, N. Benseny-Cases, G. Berruyer, D. Bugnazet, H.C.-M. Cornu, B. Fayard, E. Gagliardini, R. Hino, J. Morse, E. Papillon, E. Pouyet, C. Rivard, V.A. Solé, J. Susini and G. Veronesi, *J. Phys.: Conf. Ser.* 425, 182004 (2013).

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